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Primary Dermal Irritation Potential of Nitrosoguanidine in Female Rabbits

Earl W. Morgan, DVM, MAJ, VC
and
Don W. Korte, Jr., PhD, LTC, MSC

MAMMALIAN TOXICOLOGY BRANCH
DIVISION OF TOXICOLOGY

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September 1989

Toxicology Series: 172

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Primary Dermal Irritation Potential of Nitrosoguanidine in Female Rabbits (Toxicology
Series 172)--Morgan and Korte

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This research was conducted in compliance with the "Guide for the Care and Use of Laboratory Animals," NIH Publication No. 85-23, as prepared by the Institute of Laboratory Animal Resources, National Research Council.

This material has been reviewed by Letterman Army Institute of Research and there is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the author(s) and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense. (AR 360-5)

William C. Cole 11 Sept 89

ccr Donald G. Corby (date)
COL, MC
Commanding

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ABSTRACT

The primary dermal irritation potential of nitrosoguanidine was determined in female New Zealand White rabbits by using a modified Draize method. Two nitrosoguanidine skin application sites were evaluated on each animal following a 4-hour application to closely clipped skin. Very slight erythema was observed in 2 rabbits at test compound sites and in one rabbit at the vehicle control site by 1/2 hour after wrap removal. All rabbits had returned to normal by 24 hours after dosing. Neither edema nor any other recognizable skin reaction was detected at any time during the 14-day observation period. Nitrosoguanidine was classified as a nonirritant under conditions of this study.

KEY WORDS: Primary Dermal Irritation, Nitrosoguanidine, Propellant, Mammalian Toxicology, Rabbit, Nitroguanidine

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PREFACE

TYPE REPORT: Primary Dermal Irritation GLP Study Report

**TESTING FACILITY: US Army Medical Research and Development Command
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129-6800**

**SPONSOR: US Army Medical Research and Development Command
US Army Biomedical Research and Development Laboratory
Fort Detrick, MD 21701-5010
Project Officer: Gunda Reddy, PhD**

PROJECT/WORK UNIT/APC: 3E162720A835/180/TLBO

GLP STUDY NUMBER: 85012

**STUDY DIRECTOR: LTC Don W. Korte, Jr., PhD, MSC
Diplomate, American Board of Toxicology**

**PRINCIPAL INVESTIGATOR: MAJ Earl W. Morgan, DVM, VC, Diplomate
American College of Veterinary Preventive
Medicine, American Board of Toxicology**

PATHOLOGIST: MAJ Michael V. Slayter, DVM, VC

REPORT AND DATA MANAGEMENT:

A copy of the final report, study protocol, retired SOPs, raw data, analytical, stability, and purity data of the test compound, and an aliquot of the test compound will be retained in the LAIR Archives.

TEST SUBSTANCE: Nitrosoguanidine

INCLUSIVE STUDY DATES: 22 August - 1 October 1985

OBJECTIVE:

The objective of this study was to determine the primary dermal irritation potential of nitrosoguanidine in female New Zealand White rabbits.

SIGNATURES OF PRINCIPAL SCIENTISTS INVOLVED IN THE STUDY

We, the undersigned, declare that GLP Study 85012 was performed under our supervision, according to the procedures described herein, and that this report is an accurate record of the results obtained.

Don W. Korte 8 Sep 89

DON W. KORTE, JR., PhD / DATE
LTC, MS
Study Director

Earl W. Morgan 2 Jul 87

EARL W. MORGAN, DVM / DATE
MAJ, VC
Principal Investigator

Conrad Wheeler 5 Sep 89

CONRAD R. WHEELER, PhD / DATE
DAC
Analytical Chemist



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REPLY TO
ATTENTION OF:

SGRD-ULZ-QA

8 September 1989

MEMORANDUM FOR RECORD

SUBJECT: GLP Compliance for GLP Study 85012

1. This is to certify that in relation to LAIR GLP Study 85012 the following inspections were made:

10 May 1985 - Protocol Review
16 September 1985 - Observations

2. The institute report entitled "Primary Dermal Irritation Potential of Nitrosoguanidine in Female Rabbits," Toxicology Series 172, was audited on 31 August 1989.

Carolyn M. Lewis
CAROLYN M. LEWIS
Diplomate, American Board of
Toxicology
Quality Assurance Auditor

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Primary Dermal Irritation Potential of Nitrosoguanidine in Female Rabbits-
Morgan and Korte

INTRODUCTION

Nitrosoguanidine is a potential anaerobic degradation product of nitroguanidine (1), a primary component of US Army triple-base propellants, which is now produced in a Government-owned contractor-operated ammunition plant. The US Army Biomedical Research and Development Laboratory (USABRDL), as part of its mission to evaluate the environmental and health hazards of military-unique propellants generated by US Army munitions-manufacturing facilities, conducted a review of the nitroguanidine data base and identified significant gaps in the toxicity data (2). The Division of Toxicology, LAIR, was tasked by USABRDL to develop a genetic and mammalian toxicity profile for nitroguanidine, related intermediates/by-products of its manufacture, and its environmental degradation products.

Objective of Study

The objective of this study was to determine the primary dermal irritation potential of nitrosoguanidine in female New Zealand White rabbits.

MATERIALS

Test Substance

Chemical Name: Nitrosoguanidine

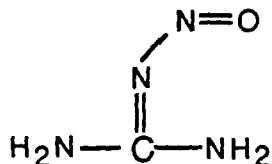
Chemical Abstracts Registry No.: 674-81-7

LAIR Code Number: TP48

Physical State: Yellow powder

Morgan and Korte-2

Molecular Structure:



Molecular Formula: CH₄N₄O

Other test substance information is presented in Appendix A.

Animal Data

Eight female New Zealand White rabbits (Elkhorn Rabbitry, 5265 Starr Way, Watsonville, CA), identified individually with ear tattoos numbered 85F176 to 85F183 inclusive, were assigned to the study. The animal weights on dosing day (16 Sep 85) ranged from 2.6 to 2.8 kg. Additional animal data appear in Appendix B.

Husbandry

The rabbits were housed individually in stainless steel, screen-bottomed, battery-type cages with automatically flushing dump tanks. The diet consisted of 150 g per day of Certified Purina Chow® Diet 5322 (Ralston Purina Company, Checkerboard Square, St. Louis, MO); water was provided by continuous drip from a central line. The animal room temperature was maintained at 17.8°C to 21.6°C with a relative humidity range of 53% to 61%, with short spikes up to 67% associated with room cleaning. The photoperiod was 12 hours of light per day.

METHODS

Group Assignment/Acclimation

Study animals were acclimated for 11 days to the study room following a 14-day quarantine by the Division of Animal Care and Services (DACS). The length of quarantine/acclimation was due to the fact animals were molting. The animals had returned to a normal condition before dosing. During this

period they were observed daily for signs of illness. They were treated prophylactically for ear mites with a single dose of Canex® and mineral oil instilled in the ears.

Test Procedures

This study was conducted in accordance with EPA guidelines (3) and LAIR SOP-OP-STX-34 (4).

The backs of 8 rabbits were close-clipped 24 hours before the actual dosing. The clipped area was divided into 4 quadrants designated I-IV (5, 6). Site I was designated a sham patch site. Sites II and III were test compound sites. Site IV was a saline control patch site. A standard dose of 0.5 g of nitrosoguanidine was moistened with enough (usually 0.5 ml) 0.9% sodium chloride solution to make a thick paste. This paste was placed on 1-inch (2.5 cm) square gauze patch that was taped to the appropriate site. Blenderm® (Medical Products Division of 3M, Saint Paul, MN), an occlusive, hypoallergenic surgical tape, was used to hold the patches in place. Vetrapp® (Animal Care Products Division of 3M, Saint Paul, MN) was then wrapped securely around the animal and taped down with Conform® elastic tape (Kendall Company, Boston, MA). The test compound was left in contact with the skin for 4 hours. At the end of the exposure period the wrapping and patches were removed, and the skin was gently wiped with a saline-moistened gauze to remove any test material remaining on the skin.

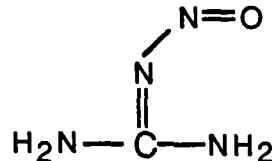
Observations

The grading and scoring for dermal reactions were performed according to Table 1. Scoring and grading for dermal irritation were performed at 30-60 minutes and approximately 24, 48, and 72 hours after removal of the patch. Observations for clinical signs were made daily from 17 September to 1 October 1985.

Duration of Study

Appendix C is a complete historical listing of study events.

Molecular Structure:



Molecular Formula: CH₄N₄O

Other test substance information is presented in Appendix A.

Animal Data

Eight female New Zealand White rabbits (Elkhorn Rabbitry, 5265 Starr Way, Watsonville, CA), identified individually with ear tattoos numbered 85F176 to 85F183 inclusive, were assigned to the study. The animal weights on dosing day (16 Sep 85) ranged from 2.6 to 2.8 kg. Additional animal data appear in Appendix B.

Husbandry

The rabbits were housed individually in stainless steel, screen-bottomed, battery-type cages with automatically flushing dump tanks. The diet consisted of 150 g per day of Certified Purina Chow® Diet 5322 (Ralston Purina Company, Checkerboard Square, St. Louis, MO); water was provided by continuous drip from a central line. The animal room temperature was maintained at 17.8°C to 21.6°C with a relative humidity range of 53% to 61%, with short spikes up to 67% associated with room cleaning. The photoperiod was 12 hours of light per day.

METHODS

Group Assignment/Accimation

Study animals were acclimated for 11 days to the study room following a 14-day quarantine by the Division of Animal Care and Services (DACS). The length of quarantine/acclimation was due to the fact animals were molting. The animals had returned to a normal condition before dosing. During this

Changes/Deviations

Dosing was delayed one week due to molting and resulting hyperemia of the skin. Final observations, weighing, and necropsy were postponed from 30 September to 1 October 1985 to accommodate military duties. These changes did not affect interpretation of study results.

Storage of Raw Data and Final Report

A copy of the final report, study protocols, raw data, retired SOPs, and an aliquot of the test compound were retained in the LAIR Archives.

RESULTS

Animals were scored for erythema and edema at each patch site. Two rabbits (85F180, 85F181) exhibited very slight erythema (dermal reaction score of 1) at test compound application sites 1/2 hour after patch removal. Since the patch site was near the stifle of both animals, the irritation could have been attributed to a mechanical rubbing of the test compound patch area. These rabbits had returned to normal by 24 hours after patch removal. Rabbit 85F179 (vehicle patch site) exhibited very slight erythema 1/2 hour after patch removal, which had returned to normal by 24 hours after patch removal. Neither edema nor any other recognizable skin reaction was detected at any time during the 14-day observation period. Results of scoring the dermal irritation potential in each rabbit are tabulated in Appendix D. Among the 8 rabbits dosed there were no gross pathological lesions attributable to the test compound or test procedures.

The pathology report is presented in Appendix E.

DISCUSSION

The modified Draize dermal irritation test as performed for this study has proven reliable for detecting nonirritating substances and severe irritants but considerably less reliable for detecting mild and moderate irritants (6). Consequently, many systems have been used to score and categorize the

dermal irritation potential of a test compound. The system used by the Toxicity Testing Program at LAIR is an adaptation of one used at the U.S. Army Environmental Hygiene Agency (7). It develops a dermal irritation index based on the peak net mean score, which is the maximum net mean score calculated during the 72-hour observation period. Nonirritating compounds have peak net mean scores of 0.0 to 0.5. Mild irritants have peak net mean scores of 0.51 to 2.0. Moderate irritants have peak net mean scores of 2.1 to 5.0. Severe irritants have peak net mean scores of 5.1 to 8.0. Nitrosoguanidine produced very slight erythema in 2 of 8 rabbits. However, there was a similiar score at a vehicle site in one animal. Consequently, the peak net mean score for nitrosoguanidine was 0.13, classifying it as a nonirritant. However, for a compound to be irritating, it must first be absorbed by the skin (8). In this assay, most of the nitrosoguanidine was still present on the skin when the patches were removed, which indicates that the nitrosoguanidine was poorly absorbed.

CONCLUSION

The test compound, nitrosoguanidine, is not a dermal irritant under conditions of this assay.

REFERENCES

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Appendix A: CHEMICAL DATA

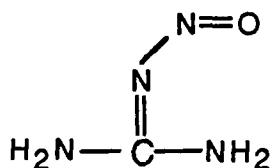
Chemical Name: Nitrosoguanidine

Chemical Abstracts Service Registry No.: 674-81-7

Lot Number: WCC-2-002

LAIR Code: TP48

Chemical Structure:



Molecular Formula: CH₄N₄O

Molecular Weight: 88

Physical State: Yellow powder

Analytical Data:

HPLC: Nitrosoguanidine was analyzed using conditions similar to those employed by Burrows *et al.*¹ Conditions were as follows: column, Brownlee RP-18 (4.6 mm x 25 cm); mobile phase, water; flowrate, 0.8 ml/min. The effluent was monitored at 255 nm. The retention times for nitrosoguanidine and nitroguanidine were 4.4 and 6 min, respectively. The HPLC data demonstrated that the nitrosoguanidine contained approximately 2.5% nitroguanidine.²

IR (KBr): 3378, 3096, 1690, 1649, 1508, 1341, 1266, 1134, 1088, 1035, 690, 668 cm⁻¹.³

Solubility:

A saturated solution of nitrosoguanidine in water was prepared at room temperature. A 1:500 dilution of this solution produced an absorbance of 0.533 units. Using an extinction coefficient of 13,305 L/moles·cm, the concentration of nitrosoguanidine in the original saturated solution was calculated to be 1.76 mg/ml.⁴

Stability:

Stable for at least 4 hours in water at room temperature.⁵

Source: Alan Rosencrance

US Army Biomedical Research and Development Laboratory
Fort Detrick, Maryland

¹ Burrows EP, Brueggeman EE, Hoke SH. Chromatographic trace analysis of guanidine, substituted guanidines and triazines in water. *Chromatog* 1984;16:494-8.

² Wheeler, CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.3, p 37. Letterman Army Institute of Research, Presidio of San Francisco, CA.

³ *Ibid.* p 30.

⁴ Wheeler CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #85-01-006, p 66. Letterman Army Institute of Research, Presidio of San Francisco, CA.

⁵ Wheeler, CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.3, p 32-36. Letterman Army Institute of Research, Presidio of San Francisco, CA.

Appendix B: ANIMAL DATA

Species: *Oryctolagus cuniculus*

Strain: New Zealand White (albino)

Source: Elkhorn Rabbitry
5265 Starr Way
Watsonville, CA 95076

Sex: Female

Age: Young adults

Animals in each group: 8 females

Condition of animals at start of study: Normal

Body weight range at dosing: 2.6 to 2.8 kg

Identification procedures: Ear tattoo, numbers 85F176 - 85F183, inclusive

Pretest conditioning:

1. Quarantine/Acclimation from 22 August - 16 September 1985
2. Animal were close-clipped and examined 24 hours before dosing.

Justification: Laboratory rabbits are a proven sensitive animal model for dermal irritation.

Appendix C: HISTORICAL LISTING OF STUDY EVENTS

<u>Date</u>	<u>Event</u>
22 Aug 85	Rabbits arrived at LAIR and were examined and caged.
23 Aug 85	Animals were weighed, and placed under a 2-week quarantine (85F165).
22 Aug -5 Sep 85	Animals were checked daily by DACS personnel
26 Aug 85	Animals were weighed and tattooed.
30 Aug 85	All rabbits were weighed and treated with Canex® and mineral oil in their ears to prevent ear mites.
5 Sep 85	Rabbits were removed from quarantine after being certified healthy by DACS Staff Veterinarian.
5 - 15 Sep 85	Animals were checked daily.
6 Sep 85	Animals were weighed.
9,15 Sep 85	Animals were close-clipped and areas marked.
16 Sep 85	Animals were weighed. Test substance was applied for 4 hours. Patches were removed and sites scored within 30 minutes.
17 Sep - 1 Oct 85	Animals were observed daily.
17 - 19, 23 Sep 85	Areas were scored at 24, 48, and 72 hours , and 7 days after exposure.
23 Sep 85	Animals were weighed.
1 Oct 85	Animals were scored, weighed, and submitted for necropsy.

Appendix D: DERMAL IRRITATION DATA

ANIMAL <u>NUMBER</u>	OBSERVATION	QUADRANT*			
		I	II	III	IV
85F176	30-60 min#	0/0†	0/0	0/0	0/0
85F177	30-60 min#	0/0	0/0	0/0	0/0
85F178	30-60 min#	0/0	0/0	0/0	0/0
85F179	30-60 min 24 hr#	0/0 0/0	0/0 0/0	0/0 0/0	1/0 0/0
85F180	30-60 min 24 hr#	0/0 0/0	0/0 0/0	1/0 0/0	0/0 0/0
85F181	30-60 min 24 hr#	0/0 0/0	0/0 0/0	1/0 0/0	0/0 0/0
85F182	30-60 min#	0/0	0/0	0/0	0/0
85F183	30-60 min#	0/0	0/0	0/0	0/0

* Quadrant I=sham; II, III=treated; IV=saline

Scores were 0/0 in all quadrants for remaining observations

† Scores are displayed as erythema/edema

Appendix D (cont.): DERMAL IRRITATION DATA**SUMMARY OF PRIMARY IRRITATION TEST DATA**

<u>Animal Number</u>	<u>30-60 min</u>			<u>24 h</u>			<u>48 h</u>		
	<u>Test*</u>	<u>Sham</u>	<u>Vehicle</u>	<u>Test</u>	<u>Sham</u>	<u>Vehicle</u>	<u>Test</u>	<u>Sham</u>	<u>Vehicle</u>
85F176	0	0	0	0	0	0	0	0	0
85F177	0	0	0	0	0	0	0	0	0
85F178	0	0	0	0	0	0	0	0	0
85F179	0	0	1	0	0	0	0	0	0
85F180	1	0	0	0	0	0	0	0	0
85F181	1	0	0	0	0	0	0	0	0
85F182	0	0	0	0	0	0	0	0	0
85F183	0	0	0	0	0	0	0	0	0
Mean	0.25	0	0.12	0	0	0	0	0	0
Net Mean Score†	0.13<			0			0		

*Test value is the larger of the scores in Quadrants II and III.

†Test Mean - (Greater of Sham or Vehicle Mean) = Net Mean Score

<The peak net mean score is 0.13; therefore, nitrosoguanidine is a **NON-IRRITANT**

Appendix E: PATHOLOGY REPORT

**LAIR Gross Pathology Report
GLP Study 85012**

Test: Primary Dermal Irritation Test

Investigator: CPT Morgan, Toxicology Branch

Test Substance: Nitrosoquanidine (CAS #674-81-7)

History: Eight female rabbits (New Zealand White), each had the compound applied to two shaved areas and control patches applied to two additional areas. Skin responses were observed at 30-60 minutes, 24, 48 and 72 hours after removing the compound.

Gross findings:

<u>PATH ACC #</u>	<u>ANIMAL ID #</u>	<u>MORPHOLOGIC DX</u>
38252	85F176	Live - Not remarkable (NR)
38253	85F177	Live - NR
38254	85F178	Live - NR
38255	85F179	Live - NR
38256	85F180	Live - NR
38257	85F181	Live - NR
38258	85F182	Live - NR
38259	85F183	Live - NR

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4 December 1985 (17 March 1986)

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Material Agency**

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Commandant

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